

SALMONID GAMETE PRESERVATION IN THE SNAKE RIVER BASIN

2007 Annual Report



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ABSTRACT

In spite of an intensive management effort, Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) populations in the Columbia River basin have not recovered and are currently listed as threatened species under the Endangered Species Act. In addition to the loss of diversity from stocks that have already gone extinct, decreased genetic diversity resulting from genetic drift and inbreeding is a major concern. Reduced population and genetic variability diminishes the environmental adaptability of individual species and entire ecological communities. The Nez Perce Tribe (NPT), in cooperation with Washington State University (WSU) and the University of Idaho (IU), established a germplasm repository in 1992 in order to preserve the remaining salmonid diversity in the region. The germplasm repository provides long-term storage for cryopreserved gametes. Although only male gametes can be cryopreserved, this project preserves the genetic diversity of these stocks and provides management options for future species recovery actions. NPT efforts have focused on preserving salmon and steelhead gametes from the major river subbasins in the Snake River basin. However, the repository is available for all management agencies to contribute gamete samples from other regions and species.

In 2007 a total of 82 viable semen samples were collected and added to the germplasm repository. This included the gametes from 66 male Chinook salmon from the Lostine River (4), Catherine Creek (13), upper Grande Ronde River (4), Lake Creek (5), Johnson Creek (17), Big Creek (2), Capehorn Creek (2), Marsh Creek (13), Elk Creek (1) and upper Salmon River (5) and; gametes from 16 male steelhead from the Tucannon River (11), Cow Creek (2), Lightning Creek (2) and South Fork Salmon River (1). Collections were smaller in 2007 due to significantly reduced fish returns and extreme fire activity during Chinook salmon spawning season. To date, a total of 2,858 Columbia River male Chinook salmon, 1,406 Columbia River male steelhead gamete samples, 22 Kootenai River male white sturgeon gamete samples and 9 Kootenai River male burbot gamete samples are preserved in the repository. Gamete collection will continue in 2008 from imperiled Chinook salmon and steelhead populations of the Snake River basin.

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The cooperation and assistance of regional Hatchery Managers is greatly appreciated including; Gene McPherson from the Idaho Department Fish and Game McCall Fish Hatchery, Greg Davis from Oregon Department of Fish and Wildlife Wallowa Hatchery, Bob Lund from the Oregon Department of Fish and Wildlife Lookingglass Hatchery, and Brent Snider from the Idaho Department Fish and Game Sawtooth Fish Hatchery.

The Nez Perce Tribe is appreciated for administrative support of this project.

INTRODUCTION

The goals of genetic conservation are to reduce the possibility of extinction and ensure the maintenance and recovery of a species as a functioning ecological unit of the environment. While preventative actions for conserving species such as habitat protection and enhancement and harvest controls are preferred, these measures frequently are not implemented until populations have reached critically low levels. Once this occurs, conservation strategies using artificial environments such as zoos, botanical gardens and live or frozen gene banks are often required (Bartley 1998). Although it is often difficult to decide when to use the more intensive actions, measures aimed at conserving the genetic diversity of a species should be implemented prior to a severe population collapse. Therefore, once a species threatened by a population collapse is identified, a combination of preventative and intensive measures should begin in order to prevent further loss of genetic diversity and preserve long-term evolutionary potential (Convention on Biological Diversity).

Nehlsen et al. (1991) concluded that least 106 major populations of salmon and steelhead on the west coast of the United States are extinct, and an additional 214 salmon, steelhead, and sea-run cutthroat trout stocks are at risk of extinction. As a first step in the recovery of anadromous fish stocks, National Oceanographic and Atmospheric Administration Fisheries (NOAA) listed 39 salmonid populations as threatened or endangered under the Endangered Species Act (ESA). Included in this list are all of the remaining wild populations of spring/summer and fall Chinook salmon and steelhead in the Snake River basin. These populations warrant protection because they possess unique genetic and life history attributes of the species and thus represent distinct population segments.

Some of this diversity is reflected by the variable size, migration and spawning timing and age structure found in different populations of these fish. For example, adult Chinook salmon migrating upstream past Bonneville Dam from March through May, and June through July are categorized as spring- and summer-run fish respectively (Burner 1951). Some streams in the Snake River are considered to have only spring Chinook, some mainly summer-run fish (e.g., those in the South Fork Salmon River), and some both forms (e.g., Middle Fork Salmon River and upper Salmon River). In most cases where the two forms coexist, spring-run fish spawn earlier and in the headwaters of the tributaries, whereas summer Chinook spawn later and farther downstream (Matthews and Waples 1991).

Snake River basin steelhead spawning areas are well isolated from other populations and include the highest elevations for spawning (up to 2,000 meters) as well as the longest migration distance from the ocean (up to 1,500 kilometers; Busby et al. 1996). Steelhead from the Snake River basin can be categorized into two major groups known as A-run and B-run fish. The A-run group passes Bonneville Dam before August 25 and the B-run group pass Bonneville after August 25 (CBFWA 1990, IDFG 1994). A-run steelhead are defined as predominately one ocean fish, while B-run steelhead are defined as two ocean (IDFG 1994). B-run steelhead tend to be larger, averaging 5-7 kilograms with maximum size up to 16 kilograms.

The recovery effort for these species has mainly focused on habitat protection and enhancement, hatchery construction, harvest controls, fish barging, and 'fish-friendly' changes in dam operation. Although these measures have been in place for decades, many populations continue to decline. Recently more intensive practices such as supplementation and captive brood rearing have begun. As opposed to conventional hatcheries, these programs utilize local stocks and attempt to minimize selection during all aspects of their life history. Although it is

too early to judge the success of these programs, the one thing that has been recognized is the importance of using local stocks for recovery.

The threat of a significant loss of genetic diversity in native fish stocks warrants the establishment of gene banks for the long-term storage of fish germplasm. A gene bank containing a collection of germplasm from multiple river basins preserves the greatest level of genetic diversity and enables recovery programs to use local stocks. This serves as insurance against population collapse and extirpation and provides options for future management programs by providing an opportunity for rebuilding lost stocks or maintaining genetic diversity caused by population bottlenecks (Ryder et al. 2000). At present, cryopreservation of male gametes is the only means of storing fish germplasm for extended periods of time. It was estimated that the storage time for fish semen held in liquid nitrogen are between 200 and 32,000 years (Ashwood-Smith 1980; Whittingham 1980; and Stoss 1983). Although preservation of the maternal nuclear DNA component has been accomplished in mammals (Rall and Fahy 1985, Fahning and Garcia 1992, Dobrinsky et al. 1991, Ali and Shelton 1993, Kono et al. 1988, Trounson and Mohr 1983, Hayashi et al. 1989), it has not been accomplished with fish. Successful development of methods to preserve female gametes is an active area of research and would greatly increase the ability to recover extinct salmonid stocks.

Cryotechnology is important in the conservation of aquatic species throughout the world (Harvey et al. 1998; Cloud and Thorgaard, 1993) and its widespread use resulted in scientific improvements enhancing its utility as a conservation tool (Cloud, 2003a; 2003b; Tiersch and Mazik, 2000; Wheeler and Thorgaard, 1991; Stoss, 1983). Using cryotechnology in a recovery program not only preserves genetic diversity for future management options, it also increases genetic diversity and reduces extinction risk in the short term by increasing the effective population size of the population (Ballou 1992). For these reasons, cryopreserved sperm has become an important part of recovery programs in the Snake River basin, especially those that fall under the Safety Net Artificial Propagation Program (SNAPP) such as the Redfish Lake Sockeye and the Grande Ronde Captive Broodstock Projects.

The Nez Perce Tribe (NPT) initiated Chinook salmon (*O. tshawytscha*) cryopreservation activities in 1992 (Kucera and Blenden 1999) in response to the severely reduced returns of adult Chinook salmon in Big Creek (a tributary of the Middle Fork Salmon River). In subsequent years, a more comprehensive gene banking effort was initiated (Faurot et al. 1998) including collections from additional Chinook spawning aggregates in the Snake River basin and collections from steelhead (*O. mykiss*) populations in the region (Armstrong and Kucera 1999). By collecting from numerous populations of spring and summer Chinook salmon and steelhead across the entire Snake River basin, we hope to preserve the greatest amount of endemic salmonid diversity.

This annual report details NPT germplasm preservation activities from 2007 and updates the status of the long-term repository.

METHODS

Description of Spawning Aggregates

The cryopreservation project managed by NPT currently seeks to preserve male spring and summer Chinook salmon and steelhead gametes in the Snake River basin (Figure 1). The large number of subbasins within this region has resulted in a genetically diverse collection of anadromous species. The following is a list of the sub-basins and locations that were sampled in 2007.

CHINOOK SALMON

Grande Ronde River Subbasin

1. Catherine Creek (collected at Lookingglass Hatchery)
2. Upper Grande Ronde River (collected at Lookingglass Hatchery)
3. Lostine River (collected at Lookingglass Hatchery)

Salmon River Subbasin

4. Lake Creek
5. Johnson Creek
6. Marsh Creek
7. Capehorn Creek
8. Elk Creek
9. Big Creek
10. Upper Salmon River (collected at Sawtooth Fish Hatchery)

STEELHEAD

Tucannon River Subbasin

11. Tucannon River (collected at Lyons Ferry Hatchery)

Salmon River Subbasin

12. South Fork Salmon River

Imnaha River Subbasin

13. Cow Creek
14. Lightning Creek

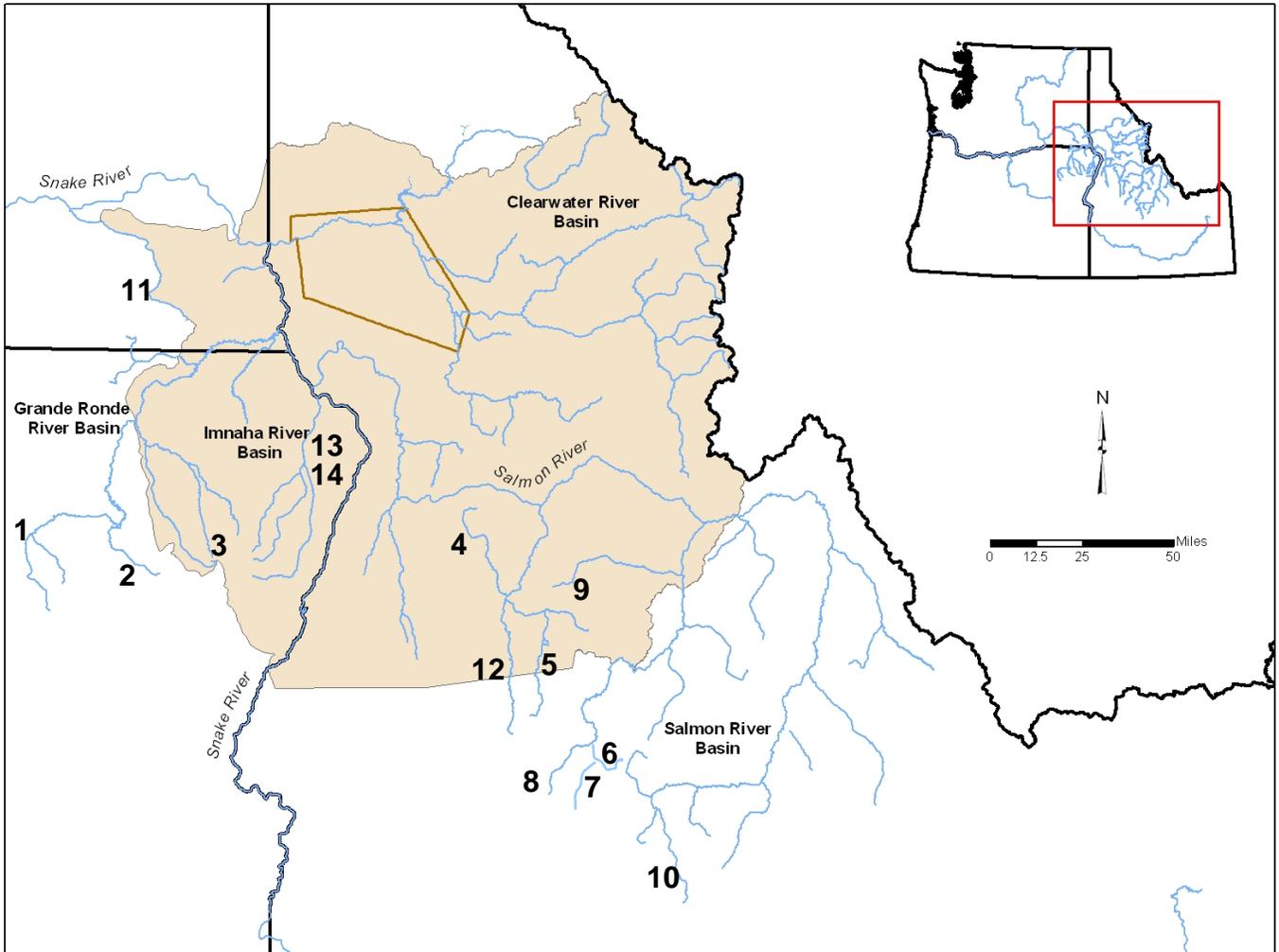


Figure 1. Map showing the Snake River basin Chinook salmon and steelhead sampling locations for 2007. The gold outlined area represents the current Nez Perce Tribe reservation boundary. The tan shaded area represents the Indian Claims Commission (ICC) area. Sample locations included; 1) Catherine Creek; 2) Grande Ronde River; 3) Lostine River; 4) Lake Creek; 5) Johnson Creek; 6) Marsh Creek; 7) Capehorn Creek; 8) Elk Creek; 9) Big Creek; 10) upper Salmon River; 11) Tucannon River; 12) South Fork Salmon River; 13) Cow Creek and; 14) Lightning Creek.

Fish Collection and Handling

Chinook salmon spawning ground surveys determined when and where in each stream the collection of adult males would be most effective. Several team members located adults and visually identified male salmon, being careful not to disturb the fish. Actively spawning females and males paired with females were avoided so as not to disrupt spawning. Males were

identified by secondary sexual characteristics such as a kype, large teeth, and a slim caudal peduncle that is not as worn as the female salmon. Personnel were instructed to stay away from any existing or active redds. A snorkeler entered the water to find solitary males, looking under cut banks, in logjams, in backwater habitats, etc. From the vantage point underwater, this person identified fish for others to collect.

All adult male salmon were collected by hand or dip net in that order of preference. Hand collections involved walking or swimming up to the identified fish and grasp the fish at caudal peduncle, putting the fish into a dip net and keeping the fish in the water, pointing upstream, until ready to place in the tank. Dip net collection involved placing several dip netters in a position downstream of the fish, being careful to avoid redds, while several upstream people slowly herd fish towards the netters. The large dip nets are held in the water in a line effectively blocking the stream until the fish swims into the net. Inadvertently caught females were immediately released from the net without ever being out of the water and the capture was recorded.

Captured fish were held in the stream while a portable tank was set up along the stream. Fish were immobilized using anesthetic so they could be handled faster and less stressfully. The anesthesia was delivered by placing the fish in a portable tank filled with 135 liters of water containing 90 mg/l of tricaine methanesulfonate (MS-222, Finquel™) anesthesia and approximately 180 mg/l sodium bicarbonate (NaHCO_3) to buffer the acidity of the MS-222. The fish was constantly monitored while in the tank and the time to sedation was noted. The sedated fish was rinsed in the fresh water of the stream and the abdomen dried to reduce water contamination prior to collecting the milt. Milt was collected in a plastic Whirl Pak bag by gently squeezing the abdomen (Figure 2).



Figure 2. Collecting milt from anaesthetized Chinook salmon.

General biological information such as fork length, mid-eye to hypural plate length, general condition and external marks were recorded following semen collection (Figure 3). Caudal fin tissue was collected and preserved in ethyl alcohol for later genetic (DNA) analysis and scales were taken for age assessment and scale pattern analysis. Stream water was gently poured over the salmon's head and gills to start the recovery from the MS-222 and reduce stress on the fish while this information was collected. Following sampling and data collection, the anesthetized salmon were immediately returned to a slow water area and assisted until it fully recovered. After the fish is released into the stream, the tank was emptied well away from the stream to prevent the release of chemicals into the stream proper.

Spring/summer Chinook salmon gametes were also collected at weirs and hatchery traps. Fish were either anesthetized by personnel working the traps or euthanized following production spawning. Milt was then collected using the standard protocol (see above).



Figure 3. Anaesthetized male Chinook salmon on portable tank for measurements.

The brood year of each sampled fish was determined initially using length data and will be modified following scale analyses if the scales provide a better estimate of age. We used the following length age relationship to determine the ages of Chinook salmon: <66 cm - age 3, 66-90 cm - age 4 and >90 cm – age 5.

In 2003 we obtained ESA section 10 permit approval to capture adult steelhead males by angling (Permit # 1134). The permit states that we were limited to artificial lures and barbless hooks. The preferred method involved locating male steelhead away from active redds and targeting these fish. At other times we fished deep holding water. Once hooked, fish were brought in as rapidly as possible, netted and held in the water until the anesthesia tank was set

up. Sperm was taken as described for Chinook salmon above. The fish were measured (fork length) and a tissue sample was taken for DNA analysis. Fish were revived by holding them in the current until they swam away. We used the following length age relationship to determine the ages of steelhead collected from the Innaha River subbasin (Little Sheep, Cow and Lightning Creeks): <64 cm - age 3 and > 64 cm – age 4. We used the following length age relationship to determine the ages of steelhead collected from the South Fork Salmon River (B-run steelhead; data from Dworshak National Fish Hatchery): <72 cm – age 3, 72 – 93 cm – age 4 and >93 cm – age 5.

Semen Handling and Cryopreservation

The amount of semen obtained varied greatly by individual fish and by species. Chinook salmon produced greater volumes of milt (averaging > 5 ml), whereas steelhead produced less (average 2-4 ml). The bags are aerated with ambient air using a foot pump then placed in an insulated cooler containing wet ice. Because it is critical to avoid placing the samples directly on the ice, newspaper was placed over the ice to insulate the samples.

Semen samples were cryopreserved at remote field sites or at the NPT McCall Field Office or Enterprise Field Office within 6 hours of collection. Sperm quality was determined by estimating the percentage of motile sperm following the addition of stream water (Mounib 1978). Samples were frozen in 0.5 ml French straws (IMV International, Minneapolis, Minnesota). Samples were stored in liquid nitrogen dewars until they were transported to Washington State University or University of Idaho for long term storage in the gamete repository (Figure 4).



Figure 4. Example of a liquid nitrogen tank used to store gametes.

Fertility trials

Annual fertility trials using sperm collected from different populations is used to assess the effectiveness of our collection, handling and storage procedures. In addition, these trials enable us to estimate the quality of our samples.

Eggs were obtained from non-ESA listed fall Chinook salmon from Bonneville Hatchery and transported to the NPT McCall Field Office. Cryopreserved sperm from Chinook salmon captured in 2007 were used to fertilize lots of approximately 100 eggs. A single 0.5 ml straw was thawed by immersing it in a 10° C water bath for 30 seconds, scraping off the ice that forms on the straw within the first 10 seconds. The straw was immediately cut open and the partially frozen milt was poured on the eggs. A sperm activator solution was added and the eggs and sperm were mixed by gently swirling the container. After one minute the activator was poured off and the eggs were rinsed twice with 10° C water. One egg lot was fertilized with fresh sperm as a control. Eggs were incubated in 100 ml glass beakers at 10 C for 14 hours. A small number of eggs were removed from the control egg lot, cleared using Stockard's solution and observed for cleavage. Embryo development at fourteen hour incubation at 10 C should be at the second cleavage stage. Once development proceeds past the 4 cell stage it becomes difficult to determine if an egg contains a viable embryo or did not begin development. Following an assessment of a portion of the control eggs, it was determined that development may have proceeded beyond the 4 cell stage. Consequently, development was arrested for all egg lots and they were cleared using Stockard's solution.

RESULTS

Gametes from 66 male Chinook salmon (Table 1) were collected and cryopreserved from 10 populations in 2007. Collections occurred from August 6 to September 6, 2007. Gametes were collected from 61 unmarked, natural-origin fish and 5 marked, hatchery-origin fish. Three males were recaptured from Marsh Creek, one adipose fin clipped fish (hatchery origin) was captured in both Lake Creek and Capehorn Creek and two Elk Creek males were captured but not sampled because they did not produce sperm. One female was accidentally captured in Marsh Creek and immediately released. Motility of the sperm ranged from 0 – 90%. Motility was estimated using water, not activator as in previous years. Sperm motility assayed with water produced variable, and often lower, estimates compared to that using activator solution (Joseph Cloud, personal communication).

Gametes from 16 male steelhead (Table 2) were collected and cryopreserved from 4 populations in 2007. Collections occurred from March 14 to May 11, 2007. Gametes were collected at Lyons Ferry Hatchery (Tucannon River steelhead), Cow Creek weir, Lightning Creek weir and by angling in the South Fork Salmon River. Motility of the sperm ranged from 0 – 90%.

2007 Chinook Salmon Gamete Collections

Lostine River

In 2007 the gametes from four male Chinook salmon were cryopreserved from fish trapped at the adult weir on the Lostine River and spawned at Lookingglass Hatchery. The

collection included gametes from two adipose fin clipped, hatchery-origin male and two unmarked, natural-origin males. Based on the length data (Appendix B), three age 4 and one age 5 fish were sampled from brood years 2003 and 2002 respectively. Collections from 1994 to 2007 have preserved a total of 174 Lostine River male gamete samples in the gene bank (Appendix A).

Upper Grande Ronde

In 2007 the gametes from four male Chinook salmon were cryopreserved from fish trapped at the adult weir on the upper Grande Ronde River and spawned at Lookingglass Hatchery. The collection included gametes from one hatchery-origin male and three unmarked, natural-origin males. Based on the length data (Appendix B), three age 4 and one age 5 fish were sampled from brood years 2003 and 2002, respectively. Collections from 2001 to 2007 have preserved a total of 59 Grand Ronde River male gamete samples in the gene bank (Appendix A).

Catherine Creek

In 2007 the gametes from 13 male Chinook salmon were cryopreserved from fish trapped at the adult weir on the Catherine Creek and spawned at Lookingglass Hatchery. The collection included gametes from three adipose fin clipped, hatchery-origin males and ten unmarked, natural-origin males. Based on the length data (Appendix B), nine age 4 and four age 5 fish were sampled from brood years 2003 and 2002, respectively. Collections from 2001 to 2007 have preserved a total of 66 Catherine Creek male gamete samples in the gene bank (Appendix A).

Imnaha River

Gametes were not collected from Imnaha River Chinook salmon in 2007. Collections from 1994 to 2007 have preserved a total of 507 Imnaha River male gamete samples in the gene bank (Appendix A). Of these, 215 were from marked hatchery-origin males and 268 were from unmarked natural-origin males.

South Fork Salmon River

Gametes were not collected from South Fork Salmon River Chinook salmon in 2007. Collections from 1996 to 2007 have preserved a total of 376 South Fork Salmon River male gamete samples in the gene bank (Appendix A). Of these, 183 were from non-ESA-listed hatchery-origin males, 85 were from ESA listed, supplementation males, and 106 were from unmarked, ESA-listed natural-origin males.

Lake Creek

In 2007 the gametes from five unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Lake Creek. One adipose fin-clipped male was captured and released without taking a sperm sample (fork length and tissue samples were obtained). Based on the length data (Appendix B), one age 3, three age 4 and one age 5 fish were sampled,

originating from brood years 2004, 2003 and 2002, respectively. Collections from 1996 to 2007 have preserved a total of 168 Lake Creek male gamete samples in the gene bank (Appendix A).

Table 1. Locations and numbers of spring and summer Chinook salmon milt samples cryopreserved in the Snake River basin in 2007.

Spawning Aggregate	Total Samples	Unmarked Fish ^a	Marked Fish ^b	Females Captured	Collection Dates	Sperm Motility (%)
Lostine River	4	2	2	0	9/5	0-90
Catherine Creek	13	10	3	0	8/29, 9/6	0-90
Grande Ronde River	4	4	0	0	9/6	0-90
Lake Creek	5	5	0	0	8/6, 13, 20	70-90
Johnson Creek	17	17	0	0	8/24, 28, 31	10-90
Big Creek	2	2	0	0	8/7, 15	unknown
Capehorn Creek	2	2	0	0	8/22	90
Marsh Creek	13	35	0	1	8/16, 21, 22	0-90
Elk Creek	1	1	0	0	8/23	0
Upper Salmon River	5	5	0	0	8/31	70-90
Totals	66	61	5	1	8/6 – 9/6	0-90

^aNon fin-clipped fish, natural-origin

^bFin-clipped or tagged fish, hatchery-origin

Johnson Creek

In 2007 the gametes from 17 male Chinook salmon were cryopreserved from fish captured in Johnson Creek. All gametes were collected from males captured at the Johnson Creek adult weir and spawned at McCall Hatchery's South Fork Salmon River facility as part of the Johnson Creek supplementation project. Based on the length data (Appendix B), one age 3, thirteen age 4 and three age 5 fish were sampled, originating from brood years 2004, 2003 and 2002, respectively. Collections from 1997 to 2007 have preserved a total of 391 Johnson Creek male gamete samples (Appendix A).

Table 2. Locations and numbers of steelhead semen samples cryopreserved from the Snake River basin in 2007.

Spawning Aggregate	Total Samples	Un-marked Fish ^a	Marked Fish ^b	Females Captured	Collection Dates	Sperm Motility (%)
Cow Creek	2	2	0	0	4/2-4/18	unknown
Lightning Creek	2	2	0	0	4/2-4/18	unknown
Tucannon River	11	11	0	0	3/14	0-90
South Fork Salmon River	1	1	0	2	4/19, 20, 5/1, 11	80
Totals	16	16	0	2	3/14 – 5/11	0-90

^aNon fin-clipped fish, natural origin

^bFin-clipped or tagged fish, hatchery origin

Big Creek

In 2007 the gametes from two unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Big Creek. Based on the length data (Appendix B), one age 4 and one age 5 fish were sampled, originating from brood years 2003 and 2002, respectively. Collections from 1992 to 2007 have preserved a total of 172 Big Creek male gamete samples in the gene bank (Appendix A).

Capehorn Creek

In 2007 the gametes from two unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Capehorn Creek. One adipose fin-clipped male was captured and released without taking a sperm sample (fork length and tissue samples were obtained). Based on the length data (Appendix B), one age 3 and one age 4 fish were sampled, originating from brood year 2004 and 2003, respectively. Collections from 1997 to 2007 have preserved a total of 36 Capehorn Creek male gamete samples in the gene bank (Appendix A).

Marsh Creek

In 2007 the gametes from 13 unmarked, natural-origin male Chinook salmon were cryopreserved from fish captured in Marsh Creek. Three males were recaptured and immediately released without taking an additional sample. One female was captured and immediately released. Based on the length data (Appendix B), two age 3, three age 4 and eight age 5 fish were sampled, originating from brood year 2004, 2003 and 2002, respectively.

Collections from 1997 to 2007 have preserved a total of 126 Marsh Creek male gamete samples in the gene bank (Appendix A).

Elk Creek

In 2007 the gametes from one unmarked, natural-origin male Chinook salmon was cryopreserved from fish captured in Elk Creek (Middle Fork Salmon River tributary). In addition, two unmarked males were captured but did not produce enough sperm for cryopreservation. Based on the length data (Appendix B), the male was age 4, originating from brood year 2003. This was the first year that gametes were collected from Elk Creek Chinook salmon.

Upper Salmon River

In 2007 the gametes from five upper Salmon River male Chinook salmon were cryopreserved from fish spawned at Sawtooth Fish Hatchery. All five were from unmarked, natural-origin males. Based on the length data (Appendix B), four age 4 and one age 5 fish were sampled, originating from brood year 2003, and 2002 respectively. Collections from 1997 to 2000 have preserved a total of 354 upper Salmon River male gamete samples in the gene bank (Appendix A). Of these, 78 were from marked hatchery fish, 28 were from marked supplementation fish and 248 were from unmarked natural fish.

2007 Steelhead Gamete Collections

Tucannon River

In 2007 the gametes from 11 natural-origin male steelhead were cryopreserved from fish spawned at Lyons Ferry Hatchery with assistance of the Washington Department of Fish and Wildlife (WDFW). Lengths were not obtained. Collections from 2005 to 2007 have preserved a total of 49 Little Sheep Creek male gamete samples in the gene bank (Appendix A).

Little Sheep Creek

No gametes were collected from Little Sheep Creek male steelhead in 2007. Collections from 1999 to 2007 have preserved a total of 464 Little Sheep Creek male gamete samples in the gene bank (Appendix A). Of these, 437 were from marked hatchery-origin fish and 27 were from unmarked natural-origin fish (Appendix A).

South Fork Salmon River

In 2007 the gametes from one unmarked, natural-origin male steelhead were cryopreserved from fish captured by angling in the South Fork Salmon River. Two females were inadvertently captured and immediately released. Based on the length data (Appendix C), the male was a two salt fish. Collections from 2003 to 2007 have preserved a total of 47 natural-origin SFSR male gamete samples in the gene bank (Appendix A).

Cow Creek

In 2007 gametes from two steelhead were sampled from fish trapped at the adult weir on Cow Creek, a tributary of the lower Imnaha River. Based on length data (Appendix C), both were 2 salt fish. Collections in 2003 and 2007 have preserved a total of four Cow Creek male gamete samples in the gene bank (Appendix A).

Lightning Creek

In 2007 gametes from two steelhead were sampled from fish trapped at the adult weir on Lightning Creek, a tributary of the lower Imnaha River. Based on length data (Appendix C), both were 2 salt fish. Collections in 2003 and 2007 have preserved a total of three Lightning Creek male gamete samples in the gene bank (Appendix A).

Grande Ronde River Chinook Salmon Captive Broodstock Project

A Grande Ronde River subbasin spring Chinook salmon captive broodstock program, co-managed by Oregon Department of Fish and Wildlife, Confederated Tribes of the Umatilla Indian Reservation and NPT, was initiated in 1995 with the collection of juvenile salmon from the Lostine River, Catherine Creek and upper Grande Ronde River. This program is an attempt to maximize the species reproductive potential and to preserve the population through use of acclimated smolt releases to return a threshold number of spawning Chinook salmon adults to the three rivers (Kline et al. 2003). Semen was cryopreserved from the male Chinook salmon in order to maintain a repository of genetic material from these captive fish. The project maintains a repository at Bonneville Hatchery. Half of the straws from each male are transported to the germplasm repository at University of Idaho as insurance against catastrophic failure at the Bonneville repository. No samples were added to the repository in 2007. The total number of samples stored in the repository from this captive broodstock project is 680. Of these, 232 were from the Lostine River, 180 were from the upper Grande Ronde River, and 268 were from Catherine Creek.

Fertility Trials

Fertility trial results

Unfortunately development of the eggs was arrested at, or just prior, to first cleavage, making it impossible to accurately assess development. This made it impossible to determine fertility rate.

Use of Cryopreserved Gametes in 2007

No gametes from the repository were requested or used in 2007.

DISCUSSION

Sustained productivity of salmonids in the Pacific Northwest is possible only if the genetic resources that are the basis of such productivity are maintained (National Research Council 1996). Because a significant portion of the genetic diversity that historically existed in the Snake River basin has already been lost, the germplasm repository is an effort to conserve the genetic diversity that remains in extant salmon and steelhead populations. In reality, the genetic diversity preserved by this project may still only represent a small portion of the total genetic diversity in the Snake River basin. Consequently, collections should continue until we can confirm that an adequate representation of the current diversity has been preserved.

Since the program was initiated in 1992, NPT has been very successful cryopreserving Chinook salmon gametes from both hatchery and natural populations. In contrast, few gametes from naturally-spawned steelhead have been collected and cryopreserved. Chinook salmon spawn in late summer during periods of low water flows, making it relatively easy to spot and capture spawning adults from natural spawning grounds. Steelhead spawn in the spring during periods of high water and inclement weather making them essentially inaccessible to capture with nets or seines. Thus, a majority of the steelhead gametes came from easily accessible hatchery-origin fish. In 2003 and 2004 we successfully collected naturally-spawning adult male steelhead using angling. In 2007 we collected gamete samples from one SFSR steelhead using this method. Steelhead spawner abundance was significantly lower in 2007 compared to 2003 and 2004. Male steelhead were often observed paired with a female on a redd making it impossible to attempt to capture the male without disturbing the spawning female. In previous years we observed large numbers of males cruising the spawning areas and could effectively target them without disrupting actively spawning females. It appears that male behavior was influenced by spawner abundance, with less aggression and competition for females in 2007.

Successful collection of steelhead gametes from Cow and Lightning Creeks was encouraging in 2007. These small tributaries in the Imnaha River subbasin are part of a large Imnaha River steelhead metapopulation that represented a significant natural-origin steelhead population in the Snake River basin. These collections were made possible with the cooperation of the NPT Lower Snake River Compensation Program that installed picket weirs in each of these creeks to estimate steelhead spawner abundance. Project personnel assisted in the monitoring of these weirs and obtained two samples from each creek. We will continue this cooperation in the future.

Tissue and scale samples were collected from nearly all fish sampled in 2007. The Chinook salmon tissue samples were sent to the Hagerman Aquaculture Research Institution and they were genetically analyzed using a standardized set of thirteen microsatellite loci (Seeb et al. 2007). A comprehensive genetic analysis report will be included in the 2008 SOW. Scale samples were archived with those of previous years. If funding becomes available we hope to analyze all of the scales in order to better understand the demographic composition of the samples in the genebank. Currently we rely on size to estimate brood year origin. Verification using scale samples will provide a greater confidence in the results.

Understanding the distribution of the samples obtained from an organism with a non-discrete generation time is critical for preserving the greatest level of diversity. This project set a goal of preserving gametes from at least 100 males per brood year for at least one generation from each spawning aggregation (Young et al, in prep). Equalizing the collection of milt from adults across an entire generation will preserve the greatest amount of genetic diversity.

However, collecting 100 samples/year for an entire generation has not been possible given the low number of returning adults and the difficulty in capturing adult males. Generally, collections ranged from 5 – 30 samples per year per spawning aggregation. Thus, it was inevitable that collections would need to continue for multiple generations in order to reach the sampling goal. For this reason we developed a method that would quantify the distribution of collections that occurred over multiple generations. This method, referred to as the Dominant Brood Year (DBY) analysis, partitioned sample collections from multiple age classes over multiple years to common brood years (Previous annual reports referred to this as Effective Brood Year analysis). A DBY is defined as the theoretical brood year an individual originated from over multiple generations. Analyzing the demographic makeup of donor fish enabled their assignment to an actual brood year. The actual brood years were assigned a specific DBY, from DBY 1 to DBY 4, corresponding to the dominant age at maturity for Chinook salmon and hatchery steelhead. Analysis indicated that it was fairly accurate going back two generations, and accurately estimated the overall distribution of samples in the genebank over short time periods (Young, 2004; Young et al. in prep). We plan to use genetic analysis to verify this method. A more comprehensive description of DBY analysis was available in previous annual reports (Young, 2005; Young, 2004).

With the exception of Imnaha River Chinook salmon and Little Sheep Creek steelhead, all Chinook salmon and steelhead populations listed in Appendix Table A1 and A2 do not have sufficient number of gamete samples (500) and will require additional sample collections beyond 2007. In addition, collections from the two populations listed above were largely hatchery-origin fish and increasing the proportion of natural-origin samples will warrant additional collections.

No requests for cryopreserved gametes were made in 2007. We recommend and support only the ethical use of cryopreserved genetic material from the germplasm repository. The judicious use of this vital genetic resource is imperative. To that end, we will provide criteria for accessing and using cryopreserved semen samples from the germplasm repository that will assist in rational use and inventory management. A form has been developed to request cryopreserved semen from the germplasm repository and is available for use (Appendix D). The semen request form's main function is for inventory management of the 0.5ml straws and 5.0 ml straws. The Snake River Germplasm Repository Committee, consisting of Tribal and University personnel, meets following a request for germplasm and decides how best to honor the request. The main decision factors are availability, scientific merit and ESA compliance.

RECOMMENDATIONS

1. Continue collecting gametes from Chinook salmon populations throughout the Snake River basin.
2. Utilize angling and provide staff at LSRCF Imnaha River tributary steelhead weirs to assist with the collection of gametes from steelhead populations throughout the Snake River basin.
3. Complete a genetic analysis of the Chinook salmon contained in the genebank and compare it to the source populations.
4. Continue tissue sample collections from all of the fish that are sampled in order to perform critical genetic analyses.
5. Research techniques to optimize 5.0 ml straw freezing and thawing protocols that will

6. Continue fertility trials on cryopreserved gametes in order to evaluate the freezing techniques.
7. Work to establish a Regional Germplasm Repository for gene conservation of imperiled fish and wildlife species.
8. Coordinate with University collaborators on female cryotechnology research.
9. Identify improved steelhead collection techniques/options.

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<http://www.nezperce.org/~dfm/Research/gametes.html>

This report and annual reports from 1997-2003 are available on the Internet through BPA Fish and Wildlife Publications at:

<http://www.efw.bpa.gov/cgi-bin/efw/FW/publications.cgi>

APPENDICIES

Appendix A. Gamete samples collected from 1992 through 2007

Table A1. Snake River basin Chinook salmon samples cryopreserved from 1992 through 2007.

Spawning Aggregate	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	Pre- 1995	Totals
Lostine River	4	16	14	39	16	19	33	18	2	3	2	3	5	170
Minam River		2	4											6
Upper Grande Ronde River	4	13	7	8	10	8	9							55
Catherine Creek	13	12	10	7	8	5	11							53
Rapid River								51	68	98				217
South Fork Salmon River		1	11	15	26	23	44	54	93	45	45	19		376
Lake Creek	5	8	20	26	32	18	28	15	6	3	4	3		163
Johnson Creek	17	31	48	60	51	58	62	35	5	17	7			374
Big Creek	2	9	6	22	31	21	50	7	0	1	6	0	17	170
Capehorn Creek	2	1	6	0	15	1	2	1	0	6	2			34
Marsh Creek	13	15	6	5	16	34	24	7	0	2	4			113
Elk Creek	1													1
Pahsimeroi River				20	15	39	50	50	31					205
Upper Salmon River	5	13	18	25	20	54	48	40	40	41	51			349
Imnaha River		20	12	25	23	7	37	71	95	79	41	33	64	507
Totals	66	141	162	252	263	286	398	349	340	295	162	58	43	2,858

Table A2. Snake River basin steelhead samples cryopreserved from 1993 through 2007.

Spawning Aggregate	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1994	1993	Totals
Tucannon River	11	16	22											38
North Fork Clearwater River						64	81	89	62					296
Selway River												5*		5
Fish Creek						3	1	1					10*	15
Grande Ronde River							1	1						2
South Fork Salmon River	1	3	2	24	17									46
Johnson Creek				1			1		2					4
Pahsimeroi River						63	60	40	47					210
Imnaha River								2						2
Little Sheep Creek		3	11	100	70	95	78	52	25	25	5			464
Cow Creek	2				2									2
Lightning Creek	2				1									1
Snake River						58	73	98	76					307
Totals	16		35	125	90	280	295	281	214	25	5	5	10	1,406

* Samples collected by the USGS/ National Biological Survey.

Appendix B. Data from Chinook salmon collected in 2007.

Table A3. Collection date, fork lengths, percent motilities and number of straws from Chinook salmon collected in 2007.

Location	Date	Fork length (cm)	Genebank #	motility assay solution	motility (%)	# of 0.5 ml straws
Big Creek	8/15/2007	91	NPT-BC01-07	-	-	20
Big Creek	8/15/2007	62	NPT-BC02-07	-	-	20
Lake Creek	8/6/2007	56	NPT-LC01-07	-	-	20
Lake Creek	8/6/2007	98	NPT-LC02-07	-	-	20
Lake Creek	8/13/2007	74	NPT-LC03-07	water	90	20
Lake Creek	8/20/2007	64	NPT-LC05-07	water	70	20
Lake Creek	8/20/2007	80	NPT-LC06-07	water	90	20
Marsh Creek	8/16/2007	88	NPT-MC01-07	water	80	20
Marsh Creek	8/16/2007	63	NPT-MC02-07	water	90	20
Marsh Creek	8/16/2007	105	NPT-MC03-07	water	0	20
Marsh Creek	8/16/2007	91	NPT-MC04-07	water	0	20
Marsh Creek	8/16/2007	98	NPT-MC05-07	water	0	20
Marsh Creek	8/16/2007	57	NPT-MC06-07	water	90	20
Marsh Creek	8/16/2007	98	NPT-MC07-07	water	0	20
Marsh Creek	8/16/2007	92	NPT-MC08-07	water	90	20
Marsh Creek	8/21/2007	84.5	NPT-MC09-07	water	90	30
Marsh Creek	8/21/2007	83.5	NPT-MC10-07	water	90	20
Marsh Creek	8/21/2007	98.5	NPT-MC11-07	water	90	20
Marsh Creek	8/21/2007	107	NPT-MC12-07	water	80	30
Marsh Creek	8/22/2007	105	NPT-MC13-07	water	0	20
Capehorn Creek	8/22/2007	70	NPT-CH01-07	water	90	20
Capehorn Creek	8/22/2007	83	NPT-CH02-07	water	90	40
Elk Creek	8/23/2007	84	NPT-EC02-07	water	0	20
Johnson Creek	8/24/2007	69	NPT-JC01-07	water	80	20
Johnson Creek	8/24/2007	87	NPT-JC02-07	water	90	20
Johnson Creek	8/24/2007	92	NPT-JC03-07	water	10	20
Johnson Creek	8/24/2007	94	NNP-JC04-07	water	70	20
Johnson Creek	8/24/2007	82	NPT-JC05-07	water	50	20
Johnson Creek	8/28/2007	77	NPT-JC06-07	water	90	20
Johnson Creek	8/28/2007	70	NPT-JC07-07	water	90	20
Johnson Creek	8/28/2007	70	NPT-JC08-07	water	90	20
Johnson Creek	8/28/2007	53	NPT-JC09-07	water	90	20
Johnson Creek	8/28/2007	72	NPT-JC10-07	water	80	20
Johnson Creek	8/28/2007	75	NPT-JC11-07	water	70	20
Johnson Creek	8/31/2007	65	NPT-JC12-07	water	70	20
Johnson Creek	8/31/2007	70	NPT-JC13-07	water	90	20
Johnson Creek	8/31/2007	72	NPT-JC14-07	water	90	20
Johnson Creek	8/31/2007	69	NPT-JC15-07	water	80	20
Johnson Creek	8/31/2007	78	NPT-JC16-07	water	50	20
Johnson Creek	8/31/2007	94	NPT-JC17-07	water	80	20
Upper Salmon River	8/30/2007	<90	NPT-US01-07	water	90	20
Upper Salmon River	8/30/2007	<90	NPT-US03-07	water	70	30
Upper Salmon River	8/30/2007	<90	NPT-US05-07	water	80	20
Upper Salmon River	8/30/2007	<90	NPT-US06-07	water	90	20
Upper Salmon River	8/30/2007	> 90	NPT-US07-07	water	90	16
Lostine River	9/5/2007	84	NPT-LR01-07	water	90	10
Lostine River	9/5/2007	86	NPT-LR02-07	water	0	20
Lostine River	9/5/2007	93	NPT-LR03-07	water	90	20
Lostine River	9/5/2007	83	NPT-LR04-07	water	0	20
Catherine Creek	8/29/2007	76	NPT-CC01-07	water	0	9
Catherine Creek	8/29/2007	81	NPT-CC02-07	water	90	10
Catherine Creek	8/29/2007	86.5	NPT-CC03-07	water	90	20
Catherine Creek	8/29/2007	100	NPT-CC04-07	water	0	20
Catherine Creek	8/29/2007	91	NPT-CC05-07	water	0	20
Catherine Creek	8/29/2007	76	NPT-CC06-07	water	0	20
Catherine Creek	8/29/2007	83	NPT-CC07-07	water	90	20
Catherine Creek	8/29/2007	78	NPT-CC08-07	water	0	14
Catherine Creek	8/29/2007	96	NPT-CC09-07	water	90	10

Location	Date	Fork length	Genebank #	Motility assay solution	Motility (%)	UI # of 0.5 ml straws
Catherine Creek	9/6/2007	81	NPT-CC10-07	water	90	20
Catherine Creek	9/6/2007	85	NPT-CC11-07	water	50	20
Catherine Creek	9/6/2007	104	NPT-CC12-07	water	0	20
Catherine Creek	9/6/2007	79	NPT-CC13-07	water	90	20
Grande Ronde River	9/6/2007	74	NPT-GR01-07	water	90	20
Grande Ronde River	9/6/2007	99	NPT-GR02-07	water	0	20
Grande Ronde River	9/6/2007	81	NPT-GR03-07	water	0	10
Grande Ronde River	9/6/2007	72	NPT-GR04-07	water	90	20

Appendix C. Data from steelhead collected in 2007.

Table A4. Collection date, fork lengths, percent motilities and number of straws from steelhead collected in 2007.

Location	Date	Fork Length	Fin Clip	Gene Bank #	Motility	# 0.5 ml straws
Tucannon River	3/20/2007		n	NPT-151-2006	90	20
Tucannon River	3/20/2007		n	NPT-152-2006	60	20
Tucannon River	3/20/2007		n	NPT-153-2006	90	20
Tucannon River	3/20/2007		n	NPT-154-2006	70	10
Tucannon River	3/20/2007		n	NPT-155-2006	90	20
Tucannon River	3/20/2007		n	NPT-156-2006	90	20
Tucannon River	3/20/2007		n	NPT-157-2006	80	15
Tucannon River	3/20/2007		n	NPT-158-2006	80	20
Tucannon River	3/20/2007		n	NPT-159-2006	80	10
Tucannon River	3/20/2007		n	NPT-160-2006	90	20
Tucannon River	3/20/2007		n	NPT-161-2006	90	15
SFSR	5/1/2007	80	n	NPT-031-05	80	30
Cow Crk, Imnaha	4/4/2007	67	n	NPT-032-05		7
Cow Crk, Imnaha	4/4/2007	63.5	n	NPT-033-05		7
Lightning Crk, Imnaha	4/5/2007	60.5	n	NPT-034-05		10
Lightning Crk, Imnaha	4/5/2007	58	n	NPT-035-05		10

Appendix D. Snake River Germplasm Repository Cryopreserved Semen Request Form



NEZ PERCE TRIBE

Department of Fisheries Resources Management

Administration • Enforcement • Harvest • Production • Research • Resident Fish • Watershed



MCCALL FIELD OFFICE

125 S. Mission St. • McCall, ID 83638

Phone: (208) 634-5290 • Fax: (208) 634-4097

Cryopreserved Semen Request Form

Name: _____

Affiliation: _____

Phone number: _____

Email address: _____

Date needed by: _____

Species/stock requested: _____ Hatchery or wild/natural: _____

Number of straws needed: _____ 0.5ml, _____ 5.0ml

Reason for request (clearly demonstrate need):

Name, address, and phone number of person that samples should be delivered to:

Please provide additional information as necessary (Annual Operating Plan, Management Plan, etc.). You will be contacted by phone or email to discuss the request and coordinate the transfer. The Nez Perce Tribe will assist in the fertilization of eggs and expects adequate monitoring of the results (percent of eggs fertilized, post-thaw sperm motility, etc.).

Signature: _____ Date: _____

Contact William Young at the above address (or by email: billy@nezperce.org) if you would like additional information about the gene bank or the request process. Management agencies in the Columbia River Basin are concerned with the inappropriate use of cryopreserved gametes and retain the right to refuse unjustifiable requests. See the Listed Stock Gamete Preservation Annual Reports or the management plan for additional information (www.nezperce.org/%7Edfrm/research/gametes.html).